SODIUM ION BINDING TO PARVALBUMIN STUDIED BY ^{23}Na NMR

A reply

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1. Introduction

We note that our earlier findings of Ca^{2+}–Na^+ competitive binding to parvalbumin, with Na^+ showing only moderately strong binding [1] are being challenged by another group [2]. These investigators find only weak Na^+ binding to parvalbumin even at reduced Ca^{2+} concentrations, and no Ca^{2+}–Na^+ competitive binding.

They suggest, as a possible explanation for this discrepancy, that the binding of Na^+ we see comes from the presence in our solutions of EGTA, which we use for calcium removal. Thus, instead of studying competitive Na^+–Ca^{2+} binding at the EF calcium-binding site, we would have been looking, according to their interpretation, at competitive Na^+–Ca^{2+} binding on EGTA molecules, themselves affixed to the protein.

However, we have been careful to monitor Na^+ binding by parvalbumin molecules retaining 1 Ca^{2+}/molecule, and there is no good evidence for EGTA-binding to such a singly-decalcified parvalbumin. In order for the analysis [2] of our results to be tenable, Ca^{2+} would have to be bound preferentially by EGTA molecules attached to the parvalbumin. Upon calcium titration, the first 2 Ca^{2+} would then become bound to these EGTA molecules, so that ~4 Ca^{2+} should be added to the apoprotein in order to restore the protein to its native state. This is clearly not the case: for our preparations of pike parvalbumin, addition of only 2 Ca^{2+}/protein molecule effects complete return to native parvalbumin [3].

Such a return to the initial native state upon recalcification is not always complete. While it has been demonstrated satisfactorily for pike parvalbumin, which we have used [3], and for carp parvalbumin [4], full renaturation does not occur necessarily with parvalbumins from other species [5]. Parello et al. [2] have been working with hake parvalbumin, for which to our knowledge this point has not been settled.

Indeed, the NMR results obtained by the two groups are quite different. Under similar conditions of temperature and concentration, we find an excess ^{23}Na linewidth of ~100 Hz [1], while the other group reports a significantly lower value of ~20 Hz [2]. This would be consistent with the above interpretation in which we have been studying binding of Na^+ to a protein close to the native state and renatured upon calcium addition [6,7] while the other group has been studying a mixture of the apoprotein and of the native protein. It is likely that the apoprotein is in a conformation quite different from that of the native protein.

We note that the negative findings [2] have been obtained in the presence of Dy^{3+} which are powerful binders and which, at the concentrations used, may very well block partially the CD and/or EF sites; this may also serve to explain why only weak binding of the Na^+ was found [2].

There is another possibility which has been discarded in [2]: they claim that we have been looking at Na^+ or Ca^{2+} binding to the protein mediated by EGTA, bound itself to the protein. When they treat apoparvalbumin with 0.5 equivalent of EGTA, in the presence of 30 mM Na^+, addition of 0.5 equivalent of Ca^{2+} leads to a considerable reduction of the ^{23}Na

linewidth. This observation points to the release of Na^+ in solution, either in the free state or bound to EGTA molecules, themselves liberated from the protein molecule(s). If indeed calcium-binding occurs with the attendant release of EGTA-bound Na^+ in the solution, reformation of protein molecules 75% decalcified (on the average) occurs with concomitant displacement of EGTA molecules. We submit then, that if as implied by this experiment, the 75% decalcified protein molecules do not have any bound EGTA, then the 50% decalcified protein we have been studying [1] a fortiori would not display any amount of EGTA-mediated Na^+ binding. The above-proposed release of EGTA upon Ca^{2+} addition could be due either to operation of an allosteric effect, or to competitive EGTA–Ca^{2+} binding.

To further discuss the results on hake parvalbumin [2], we note that the relevant equilibrium constants are: $K_d = 5 \times 10^{-9}$ M (Ca^{2+} binding to the CD site); 2×10^{-7} M (Ca^{2+} binding to the EF site) [8]; $K_d = 4 \times 10^{-7}$ M (Ca^{2+} binding to EGTA) [9]; it is somewhat surprising in view of these K_d values, which are commensurate with one another, that Parello et al. [2] do not consider likely, in the explanation of their results, the possibility of a competition between Ca^{2+} binding to EGTA and Ca^{2+} binding to the protein.

These authors [2] also refer to an observation of elution of a certain amount of EGTA (about 2 molecules/protein) during gel filtration. In the absence of any information about the procedure used, we believe it possible that such a contamination is due to a poorly-resolving column, rather than to the dissociation of EGTA–protein complexes.

To sum up, we stick to our earlier interpretation, especially in view of the experimental fact of a considerable ^{23}Na NMR line broadening with pike parvalbumin, which could not be duplicated with hake parvalbumin [2] because of the different factors discussed herein.

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